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Holborn,
London,
EC1N 2JT

(54) **Compositions containing alkaloid derivatives**

(57) A composition and method of diagnosing and for therapeutic treatment of tumors and of infectious diseases by administering effective amounts of cytostatic compositions of alkaloid compounds, their derivatives, salts of the alkaloid compounds, salts of said derivatives or mixtures thereof, The alkaloid component causes stimulation of the cellular defense mechanism, exhibits a great affinity for tumor cells with rapid accumulation in the tumor cells, interferes with cellular activity of tumor cells and cells attacked by virus and other antigens, and destroys malignant tumors and other antigen structures such as viruses, bacteria, fungal organisms. The alkaloid component can be unlabelled or labelled with radioactive isotopes. The compositions may contain materials which are fluorescent under U.V. light or compounds which absorb X-rays. In addition, the compositions can be used as an analgesic in the treatment of polyarthritis and as post-operative anti-inflammation reagent.

GB 2 110 533 A

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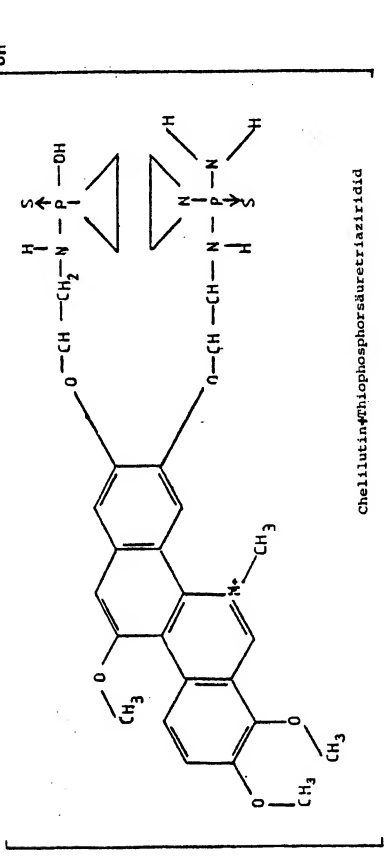
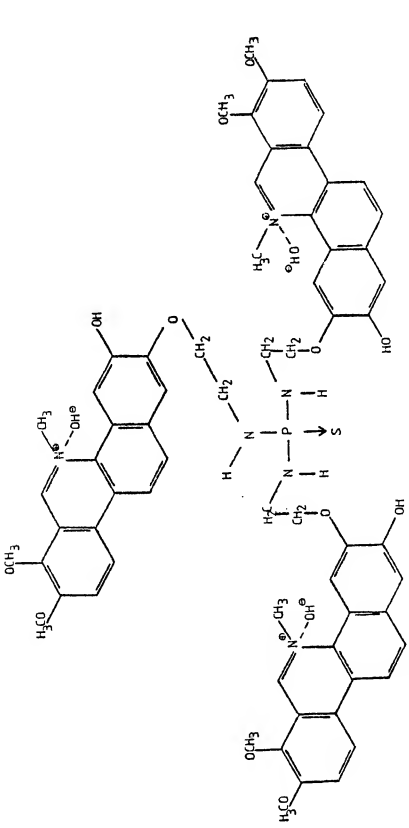
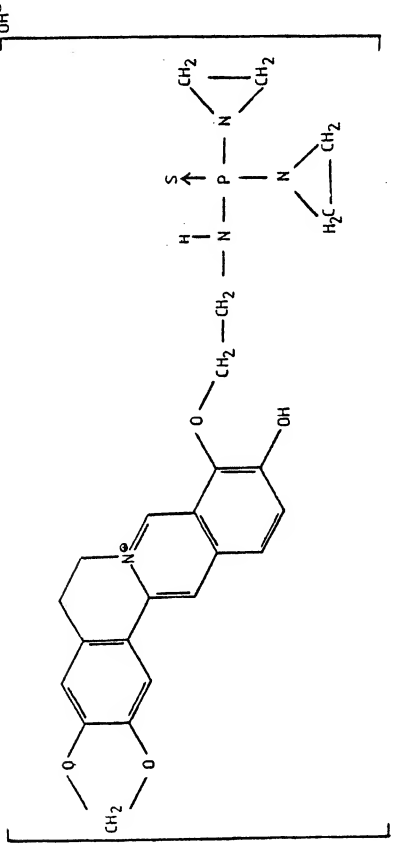


FIG. 1



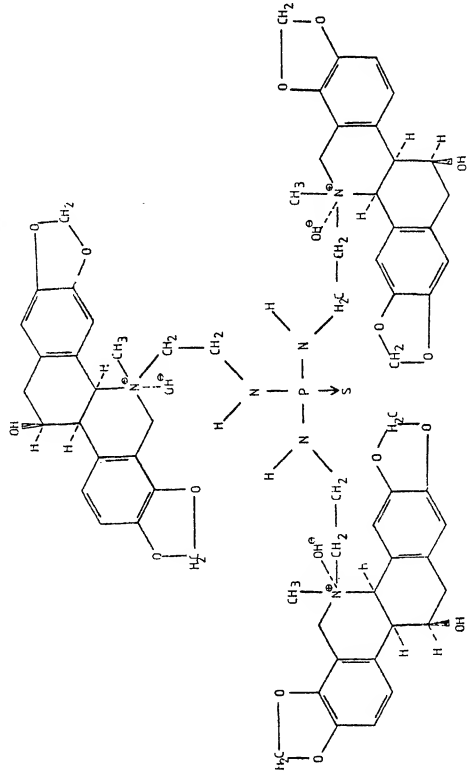
Chelerythrin-Thiophosphorsäuretriaziridid

FIG. 2



Coptisin+Thiophosphorsäuretriaziridid

FIG. 3



Chelidonin-Thiophosphorsäuretriaziridid

FIG. 4

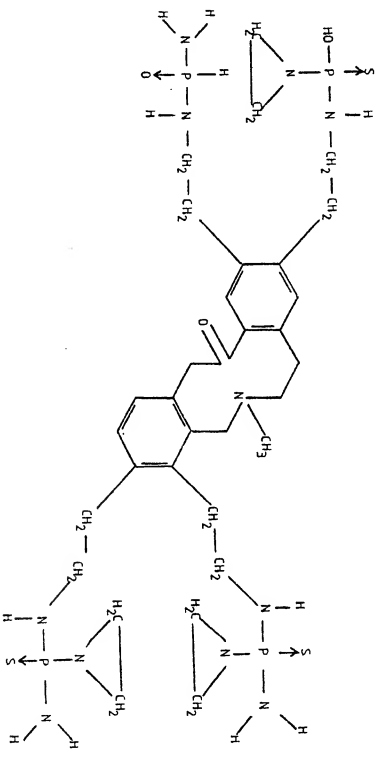


FIG. 5

Protoypin "Thiophosphorsäuretriäziridid

FIG. 6

FIG. 7

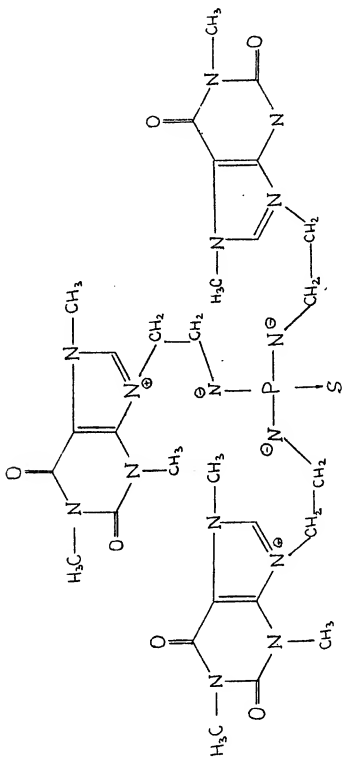


FIG. 8

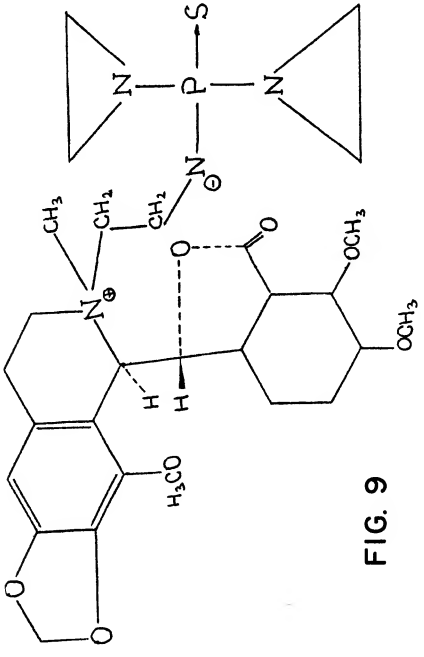


FIG. 9

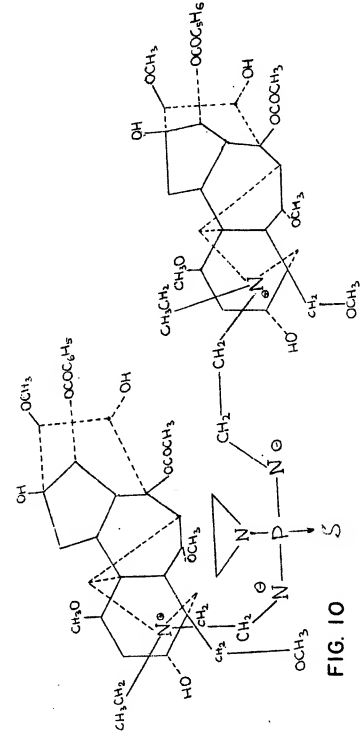


FIG. 10

OCH3
OCOC6H5
OH
OH
OCOCH3
OCH3
CH2
CH2
N
P
S
H
N
N
H
CH3O
CH3CH2
HO
N
H

FIG. 11

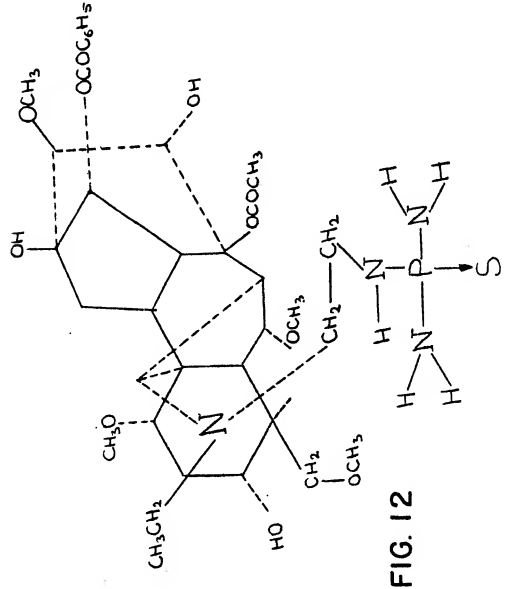

OCH3
OCOC6H5
OH
OH
OCOCH3
OCH3
CH2
CH2
CH2O
CH3CH2
HO
CH2
OCH3
N
N
H
P
S
H
H
H
H

FIG. 12

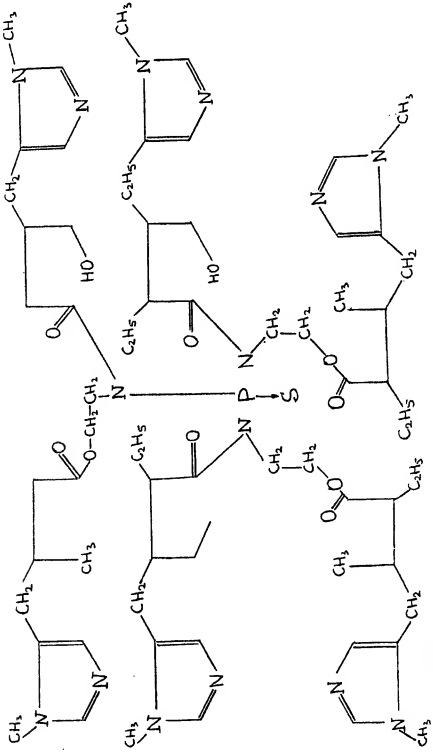


FIG. 13

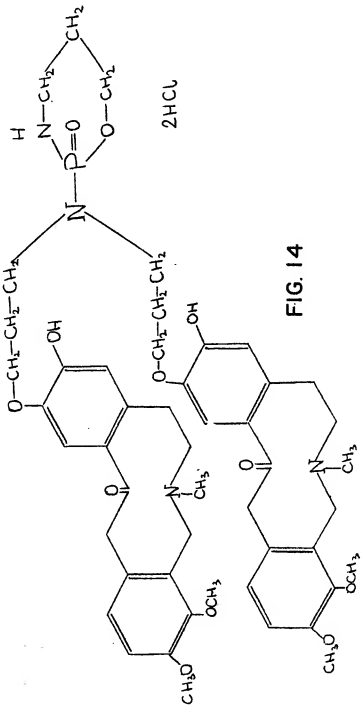


FIG. 14

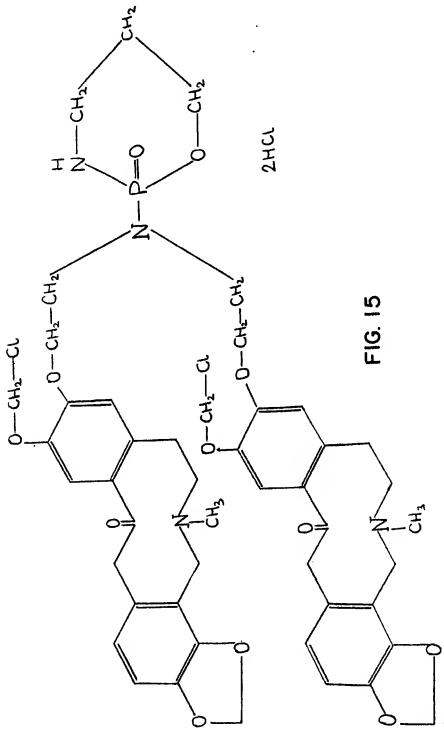


FIG. 15

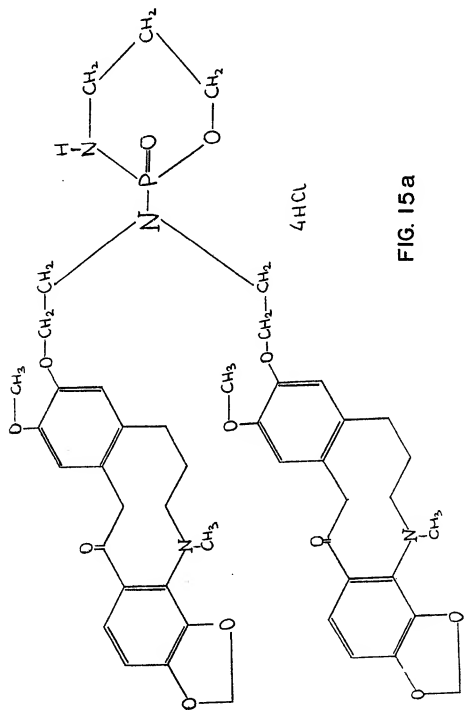
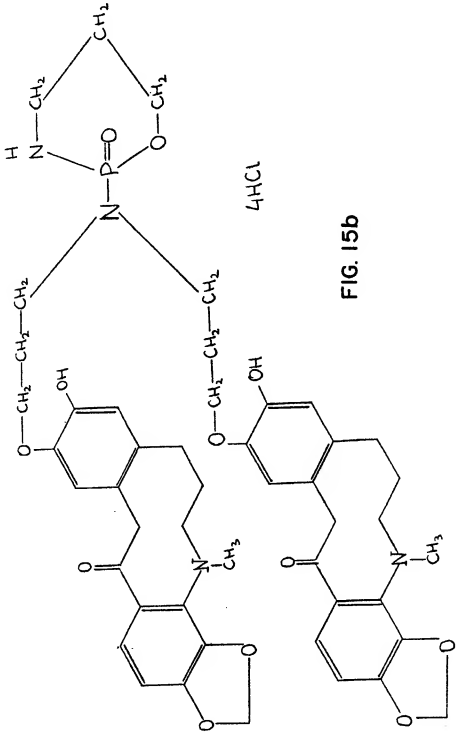


FIG. 15a



4HCl

FIG. 15b

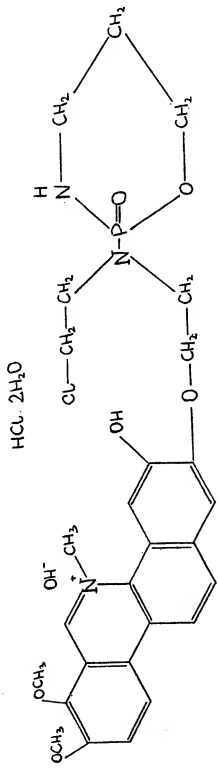

HCl. 2H2O
OH⁻
OCH3
OCH3
OH
O—CH2—CH2
Cl—CH2—CH2
N
P
O
H
N
CH2
CH2
CH2
CH2

FIG. 16

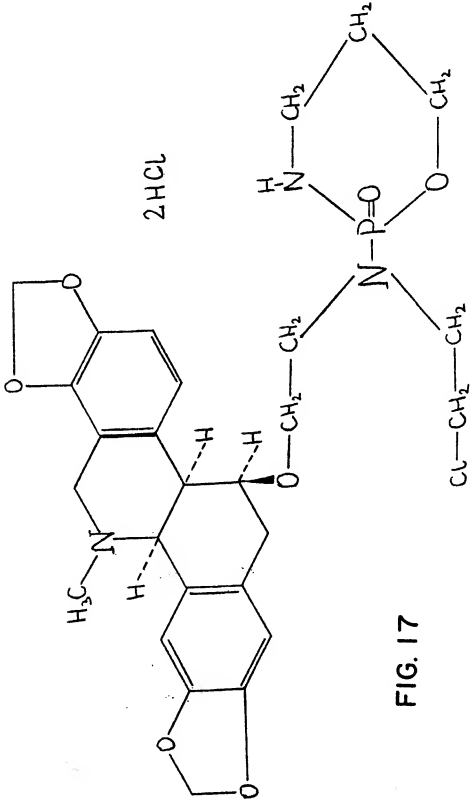


FIG. 17

SPECIFICATION

Composition and method for diegnosing end for the therapeutic treatment of tumors end/or infectious diseases of different types

The invention is directed to a composition and a method for diagnosing and for therapeutic treatment of tumors and infectious diseases. The composition is also useful as analgesics for the treatment of polyarthritis and as post-operative anti-inflammatory agents.

The invention is directed to a method of diagnosing the presence of tumor cells and for therapeutic treatment of tumors and infectious diseases and to a composition for carrying out the method. The diagnostic and therapeutic reagent is a pharmaceutically acceptable cytostatic derivative of an alkaloid which may also be carcinostatic. The terminology "pharmaceutically acceptable" means at least non-toxic to the host. The alkaloid itself can be cytostatic as well as carcinostatic. The alkaloid derivative is formed by coupling the alkaloid with a compound which may be generically referred as an alkylating reagent which will alkylate the alkaloid, which does not interfere with the cytostatic or carcinostatic property of the alkaloid or which is itself cytostatic or carcinostatic. Such alkylating reagents may contain phosphorus and/or nitrogen etoms.

The method of the invention involves administering pharmaceutically effective amounts of said derivative or water soluble pharmaceutically acceptable salts of said derivatives to the host. Administering the reegent may be undertaken by injection or intravenously or subcuteneously. The amounts of the reagent which are administered to the host depend on various factors such as body weight of the host, the stage of disease in the host and the exact nature of the disease and can range from 0.5 mg or less up to 680 mg or more. Generally, the reagents ere administered as solutions. A typical aqueous solution to be administered to the host will contein 0.5 mg of the alkaloid derivative in 1 ml of dilute saline solution. The rule in determining dosages for therapeutic purposes is an amount effective to at least retard tumor cell multiplication and, for purposes of diagnosis, to allow accumulation in the tumor tissue of amounts of the reagent sufficient to be detectable. Detection of the accumulated reagent may be undertaken by chemical means, surgery, spectroscopy and/or radiation detection methods well known in the art.

Many alkaloids are described below for use in accordance with the invention to sustain or impart the following common characteristics to the cytostatic derivatives of alkaloids used in accordance with the invention: Stimulation to the cellular defense mechanism; antagonistic to tumor cells and cells attacked by virus and other antigens; having an affinity and capacity for accumulation in tumor tissue.

Alkaloid derivatives of thiophosphoric acid exhibit pharmacological effectiveness as a sytostatic. The word "cytostatic" herein means tending to retard cellular activity and multiplication. Water-soluble salts of those alkeloid derivatives can now be made. Barberine, sanguinarine, salts of alkaloids of the large celandine can be rendered water-soluble as can salts of the bisbenzylisoquinoline-alkaloids, including curine, fangohinolin, tetrandine, pendulin, thalidasine, eporphinebenzylisoquinoline-alkaloids, e.g., thalicarpin, ibogo-alkaloids, e.g., 20-hydroxyvoacamidin, indole-indoline-alkaloids, e.g., leorosidin, lurosin, vinkaleukoblastin, leurocristin, tropolone alkaloids, e.g., colchicine, isoquinoline-alkaloids, e.g., liriodenin, O-methylatherolin, oxypurpurin, chelidonin, protopine, stylopin, allocryptopine, coptisin, chelerytrin, corysamin, chelidimerin, homochelidonin, methoxychelidonin, chellilutin, chelirubin, narciclasin, talicarpin, pakistanien, pacistanamine, pensylwenin, pensylwanamin, berberine, sanguinarine, caffeine, nitydyne, faraganin, steroid-alkaloids, indole-isoquinoline-alkaloids, e.g., 9-methoxyellipticin, ellipticin, indole-alkaloids, e.g., reserpine, quinolineindolizidin-alkaloids, e.g., campothecin, pyrolin-alkaloids, e.g., tatrofan, pyrolizidin-alkaloids, e.g., heliotrin, acridone-alkaloids, e.g., melicopin, acromycin, normelieopidin, phenanthroindolizidine-alkaloids, e.g., tylophorine, tylocrebin, imidazole-alkaloids, e.g., pilocarpine, quinolizidine-alkaloids, e.g., metrin, oxymatrin, cryptoleurin, chinazolon-alkaloids, e.g., febrifugin, benzuzepin-alkaloids, e.g., cephalotaxin, deoxyharringtonin, homcharringtonin, harringtonin and others.

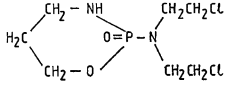
The thiophosphoric derivatives of alkaloids are also known in the form of free bases and are of interest here. Examples of such known derivatives are thiophosphoric acid-di-(ethyleneimido)-N-berberinol-ethylamide, thiophosphoric acid-tri-(n-sanguinarinol)-ethylamide as well as thiosphophoric acid amido derivatives of the total alkeloids of the condensed isoquinoline systems of the large celandine.

These thiophosphoric derivatives of alkaloids which exhibit cytostatic effectiveness are only sparingly soluble in water. In order to use the derivatives in water, which is preferred to organic solvents for the preparation of injection solutions, the derivatives must be rendered water soluble.

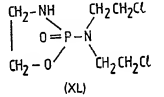
The desired water solubility can be imperted to the active alkaloid derivatives without sacrificing their cytostatic effect and without other undesirable side effects by converting the derivatives to salts of pharmaceutically acceptable acids. The alkeloid, which can also be cercinostatically effective, is coupled with a second carcinostatic agent, preferably from the group consisting of alkylantiene, antimetabolites, antibiotics, and other nitrogen- or phosphorus-containing organic compounds; and the resulting product is converted into a pharmaceutically useable selt. As used herein, the word "carcinostatic" means tending to retard epithelial malignant tumor growth and multiplication. The [illegible] thiophosphoric acid-di-(ethyleneimido)-N-

herberinol-ethylamide as well as N,N',N''-triethylene-thiophosphoramide derivatives of the condensed isoquinoline system-alkaloids are selected from the large celandine (that is, chelidonium majus L.). The aforementioned compounds have proven to be especially suitable as alkaloid components.

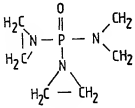
The following are especially useful as the second carcinostatic agent for the conversion:



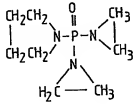
(XXXIX)



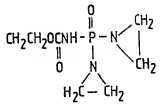
(XL)



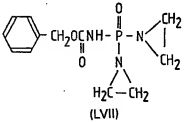
(LIV)



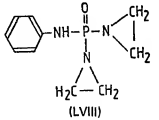
(LV)



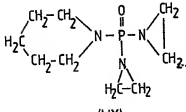
(LVI)



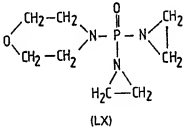
(LVII)



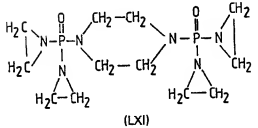
(LVIII)



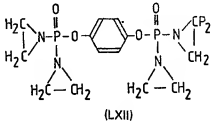
(LIX)



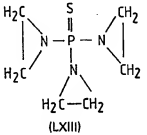
(LX)



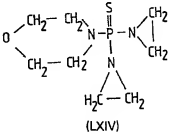
(LXI)



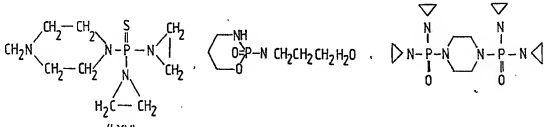
(LXII)



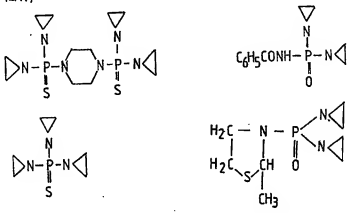
(LXIII)



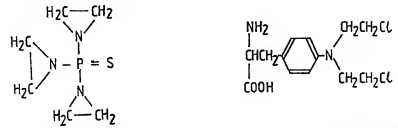
(LXIV)



(LXV)

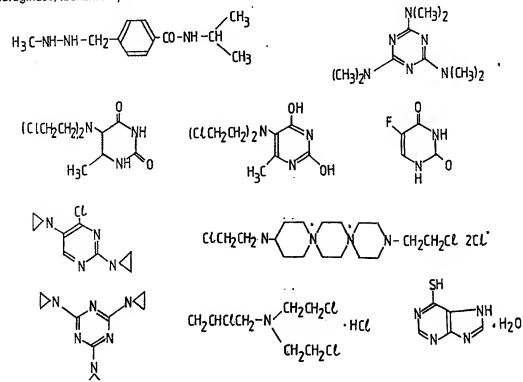


Nitrogen mustard gas, cyclophosphamide, triamcichon, chlorambucil, busulfan,



nitomin, mannitol-nitrogen mustard gas, amethopterin, 6-mercaptopurin, 5-fluorouracil, cytosine-arabinosid.

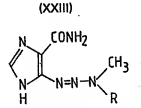
Podophyllin, actinomycin C, actinomycin D, mithramycin, mitomycin C, adriamycin, bleomycin, asparaginase, ibenzmethycin.



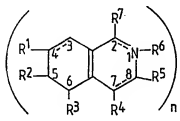

(XXII)

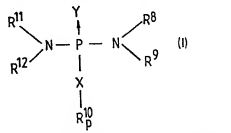

(XXIII)


(XXIV)


where R=H or CH_3.

Phosphorus derivatives of alkaloids corresponding to formula (I) below are of particular interest:



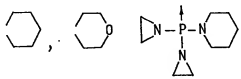

(I)

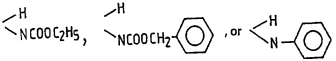
where each of R^1, R^2 and R^3 is hydrogen or methoxy, independently of each other or where R^1 and R^2 or R^2 and R^3 together can also represent a methylene dioxy group:

Where R^4 and R^5 can be H or together with the C-atoms to which they are bonded, form a completely or partially hydrated phenyl- or naphthyl group, which phenyl- or naphthyl group can be unsubstituted or substituted by methoxy, hydroxy, or dioxymethyl, when R^7 is H or =O (oxygen atom) or it is an equal ring system bound over a CH_2—CO—CH_2-chain, R^6 is CH and double bonds can be present in position 1, 2 and/or 7, 8; or

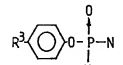
R^6 and R^7, together with the C and N-atom, to which they are bonded, form a partially hydrated benzo-or naphtho ring system, which can be unsubstituted or substituted by methoxy, oxo, methyl or dioxymethyl groups, where the C—N-bond can be missing in position 1, 2 and R^4 and R^5 mean H;

R^8+R^9 and R^{11}+R^{12} mean —CH_2—CH_2 and if Y=S, X=N and P=2, then R^8+R^9 and R^{11}+R^{12} is R^3_2—CH_2—CH_2—, —CH_2—CH_2—O—CH_2—CH_2— or —CH_2—CH_2—N—CH_2—CH_2—; when Y=S, X=N, n=2 represents

R^3_2—CH_2—CH_2—, 

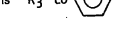
or (—C_2H_5)$_2$, 

when

Y=S, X=O, n=1 means 

when Y=O, X=N, n=1 means 

Y=O, X=N, n=2 R^3_2

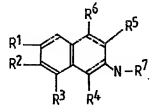
Y=O, X=O, n=1, 
and when

R^8 and R^9 each mean —CH_2—CH_2—Cl, R^{11} H and R^{10}+R^{12} mean —CH_2—CH_2— or —CH_2—CH_2—CH_2—,

The salt formation of the phosphorus derivatives of the alkaloids can be carried out with practically any pharmaceutically acceptable acid, which in itself is sufficiently water-soluble, and thus provides sufficiently water-soluble salts. For economic reasons, hydrochloric acid is preferably used.

The resulting alkaloid thiophosphoric acid amide salts do not differ in their cytostatic or in their pharmacological effectiveness from the corresponding bases. However, dosage preparation of the salts is easier and more exact due to their substantially increased water solubility. Furthermore, no disturbing side effects which can be ascribed to the organic solvents necessary for the corresponding water-insoluble bases can occur.

Salts, especially hydrochloride of berberine of sanguinarine, as well as finally the salts of the alkaloids of the large celandine and other alkaloids come into question as alkaloid salts of formula (II) (compare here also the structure formulas of Figs. 1 to 17).



The cytostetic derivative of the alkaloid can be prepared in one of two ways. The conversion of the alkaloid salts with the cytostetic medium is adventageously carried out in a solvent or solvent mixture at eleveted temperature generally at the reflux temperature. Alternatively, the alkeloid base can first be reacted with the thiophosphoric acid amide, efter which the reaction product can be converted into the salt, for example by saturating the solvent solution of the alkaloid derivative with HCl (gas and allowing the HCl saturated solution to stand. The reaction of the compound of the alkaloid with the cytostatic and with the acid is advantageously carried out in an organic solvent, where, after salt formation, the actual salt precipitates out or it can be extracted by shaking out with water or hydrous acid into the aqueous solution. Organic solvents used will vary with the alkaloid composition. By way of example, it is noted that benzene, dioxane (anhydrous), and a mixture of ether (anhydrous) and dichloroethane have been used as solvents for thiophosphoric acid-tri-(N-sanguinarinol)-ethyl amide, berberine hydrochloride and a mixture of the alkaloid extract of the great celandine and thiophosphoric acid-triethylene amide.

The alkaloid component of compositions administered in accordance with the invention may contain one or more cytostatic alkaloid derivatives, which will be explained below.

The composition of a preparation, for example, one which consists of alkaloids of Chelidonium majus L., is based on the reaction of the alkaloids with an alkylating substance, such as thiophosphoric acid triaziridide (Thio-TEPA). This substance Thio-TEPA, has three reactive groups, which either bind with the alkaloid molecules or can be substituted by OH or NH_2 groups. A whole series of different reaction products can originate in this manner, if this substance binds with an individuel, pure alkaloid. In order to study simpler reactions, individual alkaloids were mixed with Thio-TEPA, cyclophosphamide and other organic compounds in test series. In the case of chelidonin, at least 12 reaction products can be proven by thin-layer chromatography. In order to obtain an exact analysis of these reaction products some were crystallized and isolated by chromatography and subjected to elemental analysis as will be described in the description of the drawings.

Description of the drawings

The following figures of the drawings designate the possible molecular structure based on elemental analysis of the products produced in the reaction on between individual alkaloids and Thio-TEPA, cyclophosphamide and the like. In the description below, the respective alkaloid and phosphorus compounds are described after the numerical designation for the corresponding figure, followed by the actual and calculated elemental analysis for the product.

Fig. 1. Chelilutin+Thiophosphoric acid triaziridide (1-Chelilutin, 2-thiophosphoric acid triaziridide, 3-chelilutin+thiophosphoric acid triaziridide).

Calculated: C=49.44%; H=6.36%; N=11.53%; P=8.49%;
Found: C=49.41%; H=6.34%; N=10.65%; P=8.67%.

Fig. 2. Chelerythrin+Thiophosphoric acid triaziridide $C_{26}H_{29}N_4O_{15}PS$.

Calculated: C=63.45%; H=5.56%; N=6.73%; P=2.47%.
Found: C=62.69%; H=5.37%; N=5.37%; P=2.35%.

Fig. 3 Coptisin+Thiophosphoric acid triaziridide $C_{24}H_{27}N_4U_3PS$.

Calculated: C=56.02%; H=5.28%; N=10.88%; P=6.01%; S=6.23%.
Found: C=55.94%; H=5.12%; N=11.10%; P=5.89%; S=6.10%.

Fig. 4 Chelidonin+Thiophosphoric acid triaziridide $C_{68}H_{75}N_6O_{16}PS$.
Calculated: C=60.82%; H=5.79%; N=6.44%; P=2.37%; S=2.45%.
Found: C=61.41%; H=5.76%; N=5.94%; P=2.40%; S=2.39%.

Fig. 5. Protopine-thiophosphoric acid triaziridide $C_{32}H_{33}N_{11}P_4S_3$.
Calculated: C=44.59%; H=6.43%; N=17.87%; P=14.37%; S=11.16%.
Found: C=44.58%; H=6.14%; N=17.76%
C=44.72%; H=6.30%; N=17.77%; P=14.04%; S=12.71%.

Fig. 6 Reserpine+thiophosphoric triaziridide $C_{72}H_{92}N_7PSO_{16}\cdot 2H_2O$, m.p. 120—125°.
Theor.: C=59.94%; H=6.70%; N=6.79%; P=2.14%; S=2.22%.
Found: C=59.89%; H=6.62%; N=6.82%; P=2.21%; S=2.26%.

Fig. 7 Reserpine+thiophosphoric triaziridide $C_{36}H_{46}N_3O_{11}PS$.
Theor.: C=56.21%; H=6.20%; N=5.61%; P=4.14%; S=4.28%.
Found: C=56.3%; H=6.22%; N=4.11%.

Fig. 8 Caffeine+thiophosphoric triaziridide $C_{30}H_{42}N_{15}PSO_8$, m.p. 110—112°; 215—216°.
Theor.: C=46.68%; H=5.48%; N=27.22%; P=4.01%; S=4.15%.
Found: C=47.37%; H=5.44%; N=27.25%; P=4.02%; S=4.15%.

Fig. 9 Narcotine+thiophosphoric triaziridide $C_{28}H_{35}N_4PSO_7$, m.p. 225—226°.
Theor.: C=55.80%; H=5.85%; N=9.29%; P=5.13%; S=5.29%.
Found: C=55.34%; H=5.69%; N=9.52%; P=4.80%; S=5.29%.

Fig. 10 Aconitine+thiophosphoric triaziridide $C_{74}H_{106}N_8O_{22}PS$, m.p. 197—200°.
Theor.: C=60.02%; H=7.21%; N=4.72%; P=1.09%; S=2.16%.
Found C=60.02%; H=7.21%; N=4.38%; P=2.09%; S=2.16%.

Fig. 11 Aconitine+thiophosphoric triaziridide $C_{38}H_{55}N_4O_{11}PS$, m.p. 210—211°.
Theor.: C=56.92%; H=7.22%; N=6.80%; P=3.76%; S=3.86%.
Found: C=56.91%; H=7.12%; N=6.89%; P=3.60%; S=3.73%.

Fig. 12 Aconitine+thiophosphoric triaziridide $C_{35}H_{55}N_4PSO_{11}$, m.p. 190—192°.
Theor.: C=54.83%; H=7.19%; N=7.26%.
Found: C=54.83%; H=6.98%; N=8.74%.

Fig. 13 Pilocarpine+thiophosphoric triaziridide $C_{28}H_{31}N_3O_7$.

Fig. 14 Allocryptopine+cyclophosphamide (N,N-bis-(β-chloroethyl)-N,O-propylenephosphoric acid ester diamide) $C_{49}H_{65}N_4O_{12}Cl_2P$, m.p. 159—160°.
Theor.: C=8.25%; H=6.29%; N=5.58%; P=3.08%; Cl=7.06%.
Found: C=58.25%; H=6.25%; N=5.40%; P=2.53%; Cl=7.41%;
C=54.84%; H=6.16%; N=5.62%; P=2.51%; Cl=7.26%.

Fig. 15 Protopine+cyclophosphamide (N,N-bis-(β-chlorethyl)-N,O-propylene phosphoric acid ester diamide) $C_{47}H_{59}N_4O_{12}PCl_4$, m.p. 239—242°.
Theor.: C=54.24%; H=5.32%; N=5.38%; P=2.97%; Cl=13.62%.
Found: C=54.04%; H=5.25%; N=4.85%; P=2.72%; Cl=10.13%;
C=54.48%; H=5.22%; N=4.69%; P= ; Cl=9.91%.

Fig. 15[a] Protopine+cyclophosphamide (N,N-Bis'(β-chlorethyl)-N',O-propylene phosphoric acid ester diamide) $C_{47}H_{59}N_4O_{12}PCl_4$, m.p. 239—242°.
Theor.: C=54.03%; H=5.69%; N=5.36%; P=2.96%; Cl=13.57%.
Found: C=54.04%; H=5.25%; N=4.85%; P=2.27%; Cl=10.13%;
C=54.48%; H=5.22%; N=4.69%; Cl=9.91%.

Fig. 15[b] Protopine+cyclophosphamide (N,N-bis-(β-chlorethyl)-N',O-propylenephosphoric acid ester diamide) $C_{47}H_{59}N_4O_{12}PCl_4$, m.p. 239—242°.
Theor.: C=54.03%; H=5.69%; N=5.36%; P=2.96%; Cl=13.57%.
Found: C=54.04%; H=5.25%; N=4.85%; P=2.27%; Cl=10.13%;
C=54.48%; H=5.22%; N=4.69%; Cl=9.91%.

Fig. 16 Chelerythrine+cyclophosphamide (N,N-Bis-(β-chlorethyl)-N',O-propylene phosphoric acid diamide) $C_{27}H_{37}N_3O_5PCl_2$, m.p. 188—192°.
Theor.: C=49.93%; H=5.74%; N=6.64%; P=4.76%; Cl=10.91%.
Found: C=49.85%; H=5.31%; N=6.06%; P=4.95%; Cl=13.23%;
Cl=14.24%.

Fig. 17 Chelidonine+cyclophosphemide (N,N-Bis'(β-chloroethyl)-N'O-propylene phosphoric acid ester diemide $C_{27}H_{38}N_3O_7PCl_3$, m.p. 273—276°.

Theor.: C=49.82%; H=5.41%; N=6.45%; P=4.75%; Cl=16.33%.
Found: C=49.26%; H=5.07%; N=5.12%; P=3.50%; Cl=16.50%.

If e mixture of different elkeloids is mixed with Thio-TEPA, three molecules of different elkaloids may be bound to one Thio-TEPA molecule end thus e large number of components may be synthesized. In this very complex mixture of reaction products, one or more of the reaction products can be responsible for the unique biological activity. Actually, more than 50 points, fluorescent under UV-light resulted from the separation of the reaction products of Thio-TEPA and the alkaloid extract of Chelidonium majus L. by two-dimensional thin-layer chromatography. Furthermore, it could be proven that no free ethylene groups of the Thio-TEPA exist in the preparation of the reaction products produced from the alkaloid extract of Chelidonium majus L. reacted with Thio-TEPA. This proof based on chemical analysis, end biological tests to compare the lethal dose of Thio-TEPA which is 1 mg/kg body weight, wherees, e dose of 250 mg/kg body weight of the Thio-TEPA derivetive(s) of the alkaloid extract of Chelidonium majus L. is not toxic. Moreover, the preperetion hes no effect on leukemia 1210 end none on the blood picture. It can be derived from this that the ethylene groups of Thio-TEPA, which are also responsible for its toxic effect, are blocked in the reaction with alkaloids.

It is significent for diagnostic purposes that the hydrochloride of the reaction product(s) of Thio-TEPA end the elkeloid extract of Chelidonium majus L. hes the property of being yellow-green fluorescent in ultraviolet light. The excitation frequencies lie within the frequency range from 220 to 490 nm. The spectral width of the fluorescence extends from 410 nm to 665 nm, while the maximum lies at 550 nm. This large spectral width is expleined by the fect that the preparation is composed of e group of different alkaloid derivatives. The visibility limit of the fluorescence phenonemon under UV 366 nm on the plate for thin-layer chromatography lies at a dilution of 0.000007 mg/ml, 0.0000003 mg per 1 mm^2. The preperation also mainteins this property in the living body, and thus, the distribution of the preparetion could be observed in clinical experiments.

Several clinicel case studies are reported below. In each, the hydrochloride selt of the reaction product(s) of Thio-TEPA end the elkeloid extract of Cheliđonium majus L. wes employed as the preperetion for treetment and/or diegnosis.

Case 1

Patient M. B., 64 yeers old, male; diegnosis: Adenocercinome of the rectum with extensive metastatis in the LWS-area. Os il. sin. and Femur dext.

Two treatments were carried out with the preparation. Doses were 140 mg and 160 mg.

Course: Improvement of the general state, slight use of analgesics. Subjective better condition, feeling of wermth in the tumor aree rising end felling like weves.

Fluorescence factor: 6 deys after completion of the 2nd treetment cleerly demercated fluorescence in the skin eree over the tumor and its surrounding aree. Also fluorescence in a limited clinical tumor-free skin eree. These sites are identicel with the erees perceived as wermer. The greater local heat development is objectivized by bends of fever. 13 deys later, the fluorescence is much less intense appearing es irreguler spotty petterns.

Case 2

Patient R. N., 68 years old, mele; diegnosis: Bronchus-Ca II. Upper lobe. Histological: Large cell, infiltretive N. bronchi. Icterus, liver metestesis.

Three times diagnostic applications of the preparation. Total amount 130 mg. Strong fluorescence the size of a dinner plate appeared over the oxyphoid. Questionable fluorescence over the left thorax wall. One week leter: No fluorescence can be estebiished.

Case 3

Petient M. L., 72 yeers old, femele; diegnosis: Pencreas cercinoma, numerous liver metastasis. Operation: Probetoria and P.E. from a liver metastasis. Historical: Metastasis of a polymorphous cell, practically mucous forming edenocercinoma. Constent pain end extreme alkeloid consumption, Epidural catheter, Instillation v. Cerbestesin, splenchicus block. Ultresound: Liver metastesis, large pancreas. A treatment with the preperation, interrupted by giving of plecebo.

Palpatoric: Reduction in size of the left liver lobe from 5 to 3 transverse fingers under the rib case. Metastesis seems to have lessened.

After injection of the preparation, the leukocytes increased to 13000. Fluorescence present a few days after beginning of therapy, medial of the Op wound at one time 30 mm large fluorescence, was later not reproducible.

Cese 4

Petient R. M., 73 years old, femele, diegnosis: Inoperable stomech cercinome. Operetion: Probatoria and partial recticulum resection. On the evening before the operetion, 2 individual doses of

Histological: Extensive lymph angiosis carcinomatosa of the large reticulum with adenocarcinoma of the stomach. Fluorescence factor: Strong fluorescence of the resec. reticulum part. N.B.: No fluorescence of the large reticulum in the case of the healthy patient. Nodes in the mamma (Cystic mastopathy) is not fluorescent after the preparation.

Case 5

Patient W. S., 26 years old, male; diagnosis: Scar Keloid (over the xyphoid).

0.5 mg of the preparation in 1 ml 0.9% sodium chloride (physiological) solution were administered subcutaneously 50 cm removed from keloid (navel area); approximately one minute after the injection strong fluorescence of the keloid was detected continued for 2 days.

Case 6

Patient N. U., 58 years old, female, diagnosis: Mammary carcinoma. Histological: Carcinosis pleurea, multiple bone metastatis. Condition after recurring pleurae affusions, cytostatic-instillation.

2.5 mg of the preparation was injected. After a short time, fluorescence clear in the case of drain (4 RFI under OP scar) of pleurae effusion with slight fluorescence in the area of the mamma-amputation scar.

Summary of clinical results

60 patients have been treated with the preparation "Ukrain", which is a tradename for the hydrochloride salt of the reaction product(s) of thiophosphoric acid triaziridide (Thio-TEPA) and the alkaloid extract of the great celandine (Chelidonium majus L.). Further details of the preparation of this hydrochloride salt are found in my Austrian Patent No. 354,644 granted January 25, 1980, the entire disclosure of which is incorporated herein by reference. 59 of these patients were in an advanced stage of cancer.

In the course of clinical observation, it was revealed that the preparation influences tumor growth in different ways. In no case, does the preparation lead to a bone marrow depression worthy of mention.

The patients could be divided into three groups based on the stage of advancement of the disease. Group I includes 8 patients, 2 female patients with Mamma-Ca; 2 patients with malignant melanoma; 1 patient with basocellular epithelioma; 1 patient with recurrence of a cylinder cell carcinoma of the parotis; 2 patients with alleged mamma-carcinoma, one who did not agree to taking a weight specimen and the other one, on examination, suffering only multiple metastasis of an adenomacarcinoma appeared in the axillary and supraclavicular area, but in both the primary tumor could not be located. A clear remission of the tumor tissue was shown in all of the 8 cases, whereby the tumor tissue reformed partially in reverse sequence to its origin, that is to say, that those metastases which appeared last, were the first to disappear. No necrosis occurred; but next a clear demarcation of the tumor tissue appeared against the surrounding area with retrogression of the local swelling and eventually also of the existing lympho-edema with hindrance of drainage. Then, the tumor nodes slowly became smaller.

A clear effect in the form of subjective sensations was felt by these patients immediately after the first injection, such as:

Subsidal of the pain, in one case occurring 1—2 hours after the injection; feeling of warmth in the tumor, which with one patient was determined as local warming; tension and irritation in the tumor area, heat sensation, tachycardia, slight vertigo or headache; increased urine precipitation; fatigue, depression, nausea and partial depression. These symptoms did not occur at the same time; but with all patients some of the symptoms occurred in individual rhythm after each injection.

From the plots of these effects in one patient, variations in pulse, blood pressure and temperature values could be directly correlated to the occurrence of the named subjective phenomena.

Group III included 27 patients: 10 bronchial-Ca; 6 with Mamma-Ca; 2 with Ovarial-Ca; 2 with Uterus-Ca; 1 with pancreas-Ca; 2 with Rectum-Ca; 1 with stomach-Ca and one with abdominal tumor, which could not be clearly localized because of the progressed state of the primary tumor. In contrast to Group I, an effect was shown here with reference to the tumor as well as with reference to the described side effects. Even with longer treatment time and higher doses, no changes occurred which could be attributed to the preparation Ukrain.

Group II was, so-to-speak an intermediate group and consisted of 25 patients in extremely advanced state of illness: 9 with Mamma-Ca; 7 with bronchial-Ca; rectum-Ca; 2 with osteosarcoma; 1 with prostate-Ca; 1 with tyroid-Ca; 1 with colon-Ca; 1 with stomach-Ca and 1 with abdominal tumor. The effect of the preparation was very different here. With all these patients, the accompanying symptoms occurred as described in Group I patients, but not so rapidly, after extended treatment.

With these patients, the effect of the preparation on the tumor was not so clearly manifested which may be attributed to the fact that the cancer growth was very advanced. In the case of 2 patients with bronchial-Ca, a short remission of the tumor occurred. In 2 patients, a temporary cessation of growth resulted. In the case of 4 patients with Mamma-Ca, healing of the ulcerations over

freedom from pain was attributable to administration of Ukrain. In the cese of one female petient with a diffuse infiltreting thyroid gland cercinoma recurrence, the tumor changed in such e way thet it could be easily distinguished from the healthy tissue. With almost all these patients, the general condition improved notwithstending prognosis, even if the tumor growth could not be stopped.

For a better understanding of the description of the foregoing case studies, a representative case of e patient from each group is reported below.

A Group I patient:

Patient T. J., female 40 years old, diagnosis: Malignan melanoma. Histologically verified. (In 1977 a malignant melanoma on the left leg verified and operated on).

In 1980, a lymph node metastasis was found in the left groin, having a size of 5×5 cm. The patient was treated with Ukrain. In the course of a three-part treatment, she received a total of 680 mg.

During the first treatment she sustained temporary depression, muscle pain and generally poor feeling, as well as en increase in lymph node metastasis lesting 10 deys. After the second treetment the lymph node wes only the size of e welnut end after the third treetment it was the size of a bean. It was hard and not well pelpable. Her general condition was good.

A Group II patient:

Patient H. G., female, 63 years old, diagnosis: Mamma carcinoma on the left side with supraclavicular lymph node metastasis end bone metestetis. Prior systemetic therapy cerried out:

Nolvadex from June 1977 to January 1978;

Polychemotherapy with Endoxan, Methotrexate, Flour-Uracil and Prednisolone, later combination therapy with Adriblastin-Endoxan from March 1978 to April 1979. Elipten (Aminoglytethimide) from April 1979 to January 1980.

Therapy with Ukrain began on February 29, 1980. At the beginning of therapy, a 5×5 cm large ulcereted supraclevicular metastasis was found. Subjective feeling of pressure in the area of the eye as well as feeling of stress in the left supraclavicular were recorded. The therapy was begun with 2.5 mg. Right at the beginning of the therapy, the petient noticed a feeling of warmth in the area of the left side of the body, end under the supraclavicular tumor. This feeling of warmth did not reoccur. No more ulcerations were visible in the left supraclavicular after 6 injections. However, the tumor itself was unchanged. Stressful feelings end the feeling of pressure in the area of the eyes that existed for two years disappeered. During the last check on April 8, 1980, the patient was free of such subjective complaints. She complained of moderate weight loss. Local li. supraclavicuier was unchanged. No ulcerations could be proven at that time. No pathological laboratory findings (blood picture, SMA, serum calcium) could be recorded. No worsening side effects could be determined.

A Group III petient:

Patient K. G., female, 58 years old, diagnosis: Adeno-solid ovarian carcinoma, operatively removed. Prior systemetic therapy cerried out: Endoxan 1200 mg per infusions. Cobalt radiation 4500 rd/g.

Prophylactic therapy with Ukrain was undertaken. No side effects or subjective perceptions during the treatment such as vertigo, temperature increase, tachycardia or depression were experienced. The patient's general feeling, condition, and appetite improved. One month after the beginning of the therapy a node was found in the left breast; and one week later a mammectomy was carried out eccording to Patey. The histology showed a partially necroticized solid cercinoma. The treatment with Ukrain was discontinued.

To determine the effect of the mechanism of therapeutic and diagnostic capacity of Ukrain, studies were carried out concerning the immuno-stimulating effect of Ukrain in vitro in the so-called *lymphocyte-transformation test*, and results of the studies are reported in the following tables. Generally, lymphocytes are known to be immunocompetent when they are capable of recognizing the specific antigen, or if they are capable of reacting with it. Such cells are called immunocytes and their successors are called immunoblasts.

The formation of immunoblasts can also be carried out in vitro. The immunoblasts have a size of 20—30 μ, whereas, normal lymphocytes have e size of only 5—15 μ. In the following immunological tests, isolated lymphocytes of healthy humans end of guinea pigs were used for the blast transformation after the addition of Ukrain. A mixture of the compound known under the tradename Ficoll 4000 and EDTA was used for the isolation (0.9 EDTA+0.1 Ficoll 4000).

The isolated lymphocytes were grown in vitro in Parker solution, with an addition of 1.6 μg, 0.16 μg and 0.016 g. At the same time lymphocytes were bred in Parker solution with 5 μg/ml of the unspecific stimulator phytohemaglutinin (PHA) and without an additive as a control run. In order to avoid solonization of bacteria, effective amounts of antibiotics, penicillin, streptomycin and nystatin, were added. The cultures were incubated at 37°C end the number of transformed lymphocytes wes counted daily under the microscope for 3 days.

100 lymphocytes were counted out in each preparation and those lymphocytes which were larger than 15 μ (from humans) and larger than 20 μ in the case of guinea pigs, were evaluated as

being transformed. In all cases, before the beginning of breeding, the number of transformed cells was not larger than 10.1%. The tests were carried out with the lymphocytes of 10 healthy humans and 10 healthy guinea pigs.

Since in the case of a cure with Ukrain the average single dose amounts to 10 mg (the mean individual dose of Ukrain used in these experiments being 16 mg), the dose of Ukrain was actually 1/10000; 1/100000 and 1/1000000 of the amount. The results are summarized in the following Tables I and II:

Table I

Percentage of transformed guinea pigs lymphocytes.

No.	Culture-medium with additive	Breeding time 24 hrs.	48 hrs.	72 hrs.
1.	1.6*	48.6	45.6	40.2
	0.16*	42.3	29.3	28.5
	0.016*	35.6	33.6	25.0
	PHA	32.2	28.3	27.9
	Without stimulator	19.2	15.3	1.8
2.	1.6*	53.9	30.2	18.3
	0.16*	48.7	32.2	32.0 (?)
	0.016*	27.3	30.2	18.5
	PHA	20.8	17.2	17.5
	Without stimulator	13.3	12.2	19.2 (?)
3.	1.6*	50.2	48.3	42.8
	0.16*	49.3	51.1	39.9
	0.016*	41.0	29.3	30.1
	PHA	32.5	29.8	22.3
	Without stimulator	18.0	11.2	10.3
4.	1.6*	55.6	42.3	
	0.16*	50.8	40.7	43.8
	0.016*	41.2	38.5	39.5
	PHA	33.2	27.0	18.0
	Without stimulator	18.2	18.0	16.2
5.	1.6*	51.3	43.5	31.5
	0.16*	48.2	33.2	33.5
	0.016*	39.9	31.5	30.2
	PHA	33.4	20.3	11.3
	Without stimulator	12.5	12.5	8.4
6.	1.6*	58.5	57.9	41.8
	0.16*	48.3	50.0	43.2
	0.016*	40.1	35.2	30.0
	PHA	30.2	22.1	17.3
	Without stimulator	19.0	18.2	13.5
7.	1.6*	43.3	41.2	39.8
	0.16*	42.3	36.6	36.2
	0.016*	41.2	37.2	35.5
	PHA	33.3	33.4	27.2
	Without stimulator	15.1	16.1	13.2
8.	1.6*	48.7	45.1	40.3
	0.16*	43.1	39.2	31.1
	0.016*	35.6	29.8	24.2
	PHA	31.3	27.0	24.3
	Without stimulator	12.2	10.1	10.5

Table I (cont.).

9.	1.6*	53.3	51.5	41.2
	0.16*	49.6	47.2	42.2
	0.016*	39.2	36.7	34.6
	PHA	38.3	32.8	25.2
	Without stimulator	18.1	16.7	11.3
10	1.6*	56.2	53.6	40.7
	0.16*	49.3	42.8	34.5
	0.016*	39.8	37.3	28.3
	PHA	34.2	33.2	27.5
	Without stimulator	12.0	10.2	8.4

*Amount of Ukrain in units of μg/ml.

Table II

Percentage of transformed human lymphocytes.

No.	*Culture-medium with additive*	*Breeding time 24 hrs.*	*48 hrs.*	*72 hrs.*
1.	1.6*	47.2	43.4	41.2
	0.16*	43.8	39.2	38.6
	0.016*	39.6	36.7	35.2
	PHA 5 μg/ml	36.6	24.8	20.8
	Without stimulator	11.1	9.2	9.0
2.	1.6*	49.9	41.2	37.2
	0.16*	43.2	40.3	33.2
	0.016*	38.6	36.2	26.8
	PHA 5 μg/ml	31.2	28.0	21.2
	Without stimulator	18.4	14.1	8.4
3.	1.6*	19.1	18.8	16.2
	0.16*	19.5	18.2	15.3
	0.016*	14.2	15.3	13.2
	PHA 5 μg/ml	33.1	28.2	14.1
	Without stimulator	10.7	9.3	15.3
4.	1.6*	43.9	42.8	39.4
	0.16*	41.2	40.6	41.2
	0.016*	33.6	32.1	31.2
	PHA 5 μg/ml	40.2	33.6	28.3
	Without stimulator	13.6	12.3	10.8
5.	1.6*	53.3	51.5	47.8
	0.16*	48.6	43.6	41.0
	0.016*	37.2	37.8	36.2
	PHA 5 μg/ml	36.4	28.5	25.4
	Without stimulator	15.3	14.4	12.8
6.	1.6*	55.5	53.4	51.5
	0.16*	51.2	49.1	47.3
	0.016*	43.2	36.2	36.0
	PHA 5 μg/ml	37.1	36.2	34.7
	Without stimulator	19.3	18.4	16.5
7.	1.6*	48.6	46.8	42.5
	0.16*	47.2	45.2	24.3
	0.016*	39.8	32.0	21.2
	PHA 5 μg/ml	30.1	26.8	19.0
	Without stimulator	14.7	13.1	8.5

Table II (cont.).

8.	1.6*	52.3	37.4	21.8
	0.16*	48.3	28.3	19.3
	0.016*	41.2	19.4	17.4
	PHA 5 μg/ml	37.3	19.5	20.1
	Without stimulator	9.0	8.3	8.2
9.	1.6*	47.2	32.8	29.1
	0.16*	43.4	36.4	34.2
	0.016*	38.6	25.0	20.0
	PHA 5 μg/ml	36.6	28.6	21.4
	Without stimuletor	15.3	13.3	10.7
10	1.6*	48.3	37.4	33.3
	0.16*	43.4	41.2	36.8
	0.016*	37.1	36.2	29.1
	PHA 5 μg/ml	30.0	19.4	18.4
	Without stimuletor	9.3	9.6	5.3

*Amount of Ukrain in units of μg/ml.

In the test a stetistically significant difference was shown between the number of transformed lymphocytes of humans and of enimels and of control groups absent Ukrein. The conclusion can be drawn from this that the preparation Ukrain has en immunological effectiveness and stimulates the humen defense mechanism. Cleer transformation of lymphocytes did not occur in only one out of 10 cases (see Table II, No. 3, where human lymphocytes were tested. In the case of patient no. 3, under certain circumstences es "in vitro equivelent" could exist for the clinicel phenomenon, in which individual patients showed no reection et all to Ukrain).

Although no clear lymphocytes transformetion wes effected by Ukrein in the case of test person no. 3, e higher lymphocyte trensformation wes obtained with all other patients than with PHA.

Finally, the conventionel Agergel electrophoresis using 0.05 mg Ukrein, was run on Agargel (Corensen buffer+NaCl) and wes put into a small notch and lasted 4 hours at 70 volts and 2 mA, a filter peper was pressed on the surface, the stert and the polarity were marked; a fluorescent bend directed to a negative pole was defected under UV-lamp. The fluorescent fractions were positively charged.

It is epperent from the preceding that the subject preperetions or compounds described at the beginning of this specification can be used for diagnosing as well as for the therepeutic treetment of tumors of all types, and also for diegnosing end treeting infectious diseases. According to the invention, the fluorescent effect of the Ukrain preparations is of greet significence. For diegnosis, it is thus possible for rapid early detection, efter the injection of the preperetion(s), by virtue of its accumulation in the diseased tissue area. Rapid and reliable diagnosis can be besed on thet accumulation by observing decomposition end the rete of decomposition of the injection fluid in the injected erea end/or by observing the localization of seme in the diseased area, for example, in the tumor tissue. This observation extends to all endoscopic methods including bronchoscopy, mediestinoscopy, thorascopy, esophagoscopy, Otorhinoscopy, laryngoscopy, rectoscopy, proctoscopy among others, where for example, cold light is replaced by UV-light.

In this connection, the preperetion may be redioactively labelled with radioactive isotopes, and then the diagnostic method includes measuring radioectivity of the accumulated redioectively lebelled preperation in a known manner with suiteble detectors; end accumulation can be recorded for example with gamma-ray scanners. In eddition to labelling of the described preparetions with fluorescent meterials and radioactive isotopes, the preparetions may be doped with radiation-absorbing compounds or radicals, so that eccumulation of the doped preperetion mey be detected in a certain tissue area due to the increesed ebsorption of X-radietion there.

It is especially noteworthy in this connection that in the case of very meny patients, infections disappeared after the first Ukrein injection. Among them were stubborn mycosis which previously had not responded to any therepy, cases of chronic bronchitis and throet Inflammetions of unclear genesis, as well as other viral and bacterial diseases, according to the treating physicians. Thus, the previous results also suggest the stimuletion of lymphocytes to immunocytes by means of the preparation; such stimulation is equivalent to the production of the so-called killer-cells which destroy the affected tissue or the tissue foreign to the body, or virus which has penetrated healthy cells, and/or bacteria, fungi, and the like. Therefore, the healing of tumor diseases, as well as viral diseases, bacterial diseases and [illegible] and in addition a healing of polyarthritis can be realized.

Claims

1. A method of treating a diseased mammal, said mammal diseased by the multiplication of tumor cells or by the multiplication of foreign organisms, comprising administering to said diseased mammal a pharmaceutically effective amount of a pharmaceutically acceptable cytostatic alkaloid derivative, said pharmaceutically acceptable cytostatic alkaloid derivative accumulating in said tumor cells or said foreign organisms, and at least retarding the multiplication of said tumor cells or said foreign organisms.

2. The method of Claim 1, including destroying said tumor cells or said foreign organisms.

3. The method of Claim 1, wherein said pharmaceutically acceptable cytostatic alkaloid derivative is a water-soluble salt.

4. The method of Claim 1, wherein said pharmaceutically acceptable cytostatic alkaloid derivative is administered intravenously.

5. The method of Claim 1, wherein said pharmaceutically acceptable cytostatic alkaloid derivative is administered by injection.

6. The method of Claim 1, wherein said pharmaceutically acceptable cytostatic alkaloid derivative is a pharmaceutically acceptable cytostatic alkaloid extract of Chelidonium majus L.

7. The method of Claim 1, wherein said pharmaceutically acceptable cytostatic alkaloid derivative is derived from an alkaloid selected from the group consisting of chelilutin, chelerythrin, coptisin, chelidonin, protopine, reserpina, caffeine, narcotine, aconitine, allocryptopine, protopine, and admixtures thereof.

8. The method of Claim 7, wherein said pharmaceutically acceptable cytostatic alkaloid derivative is derived from an admixture of said alkaloids.

9. The method of Claim 1, wherein said pharmaceutically acceptable cytostatic alkaloid derivative is derived from chelilutin.

10. The method of Claim 1, wherein said pharmaceutically acceptable cytostatic alkaloid derivative is derived from chelerythrin.

11. The method of Claim 1, wherein said pharmaceutically acceptable cytostatic alkaloid derivative is derived from coptisin.

12. The method of Claim 1, wherein said pharmaceutically acceptable cytostatic alkaloid derivative is derived from chelidonin.

13. The method of Claim 1, wherein said pharmaceutically acceptable cytostatic alkaloid derivative is derived from protopine.

14. The method of Claim 1, wherein said pharmaceutically acceptable cytostatic alkaloid derivative is derived from reserpina.

15. The method of Claim 1, wherein said pharmaceutically acceptable cytostatic alkaloid derivative is derived from caffeine.

16. The method of Claim 1, wherein said pharmaceutically acceptable cytostatic alkaloid derivative is derived from narcotine.

17. The method of Claim 1, wherein said pharmaceutically acceptable cytostatic alkaloid derivative is derived from aconitine.

18. The method of Claim 1, wherein said pharmaceutically acceptable cytostatic alkaloid derivative is derived from allocryptopine.

19. The method of Claim 1, wherein said pharmaceutically acceptable cytostatic alkaloid derivative is derived from protopine.

20. A method of diagnosing a diseased mammal, said mammal diseased by the multiplication of tumor cells comprising administering to said diseased mammal a pharmaceutically effective amount of a pharmaceutically acceptable cytostatic alkaloid derivative, said pharmaceutically acceptable cytostatic alkaloid derivative accumulating in said tumor cells; and detecting that pharmaceutically acceptable cytostatic alkaloid derivative which has accumulated in said tumor cells.

21. The method of Claim 20, wherein said pharmaceutically acceptable cytostatic alkaloid derivative is administered by injection.

22. The method of Claim 20, wherein said pharmaceutically acceptable cytostatic alkaloid derivative is administered intravenously.

23. The method of Claim 20, wherein said pharmaceutically acceptable cytostatic alkaloid derivative is a pharmaceutically acceptable alkaloid extract of Chelidonium majus L.

24. A pharmaceutically acceptable cytostatic alkaloid derivative for use in treating or diagnosing a mammal diseased by the multiplication of tumor cells or by the multiplication of foreign organisms.

25. A composition for use in treating a diseased mammal according to the method of any one of Claims 1—23.

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